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Effects of elevated nitrogen deposition on soil microbial biomass carbon in major subtropical forests of southern China

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Abstract The effects of elevated nitrogen deposition on soil microbial biomass carbon (C) and extractable dissolved organic carbon (DOC) in three types of forest of southern China were studied in November, 2004 and June, 2006. Plots were established in a pine forest (PF), a mixed pine and broad-leaved forest (MF) and monsoon evergreen broad-leaved forest (MEBF) in the Dinghushan Nature Reserve. Nitrogen treatments included a control (no N addition), low N (50 kg N/(hm²·a)), medium N (100 kg N/(hm²·a)) and high N (150 kg N/(hm²·a)). Microbial biomass C and extractable DOC were determined using a chloroform fumigation-extraction method. Results indicate that microbial biomass C and extractable DOC were higher in June, 2006 than in November, 2004 and higher in the MEBF than in the PF or the MF. The response of soil microbial biomass C and extractable DOC to nitrogen deposition varied depending on the forest type and the level of nitrogen treatment. In the PF or MF forests, no significantly different effects of nitrogen addition were found on soil microbial biomass C and extractable DOC. In the MEBF, however, the soil microbial biomass C generally decreased with increased nitrogen levels and high nitrogen addition significantly reduced soil microbial biomass C. The response of soil extractable DOC to added nitrogen in the MEBF shows the opposite trend to soil microbial biomass C. These results suggest that nitrogen deposition may increase the accumulation of soil organic carbon in the MEBF in the study region.

Keywords nitrogen deposition, microbial biomass, extractable dissolved organic carbon (DOC), subtropical forest

1 Introduction

Nitrogen input has doubled since the early 20th century due to increasing human activities, fertilizer use and fossil fuel combustion. Nitrogen deposition in areas developed by industry and agriculture is clearly higher than in the undisturbed regions (Vitousek et al., 1997), especially in America and Europe. For instance, the current proportion of nitrogen deposition increased 10–20 times over previous levels in northeastern America (Magill et al., 1997) and N deposition is over 25 kg/(hm²·a) in the developed regions where animal husbandry and industry are located in Europe (van Breemen and van Dijk, 1988). The increase in nitrogen deposition has resulted in nitrogen saturation in many terrestrial ecosystems and affects forest ecosystems extensively (Boxman et al., 1998). Increasing nitrogen deposition can even become the dominant reason for degradation of forests in some regions of Europe and America (Boxman et al., 1998). Recent studies indicate that N deposition clearly affects ecological processes such as soil respiration, soil microbial activity, soil pH, soil nutrient elements and litter decomposition (Mo et al., 2004). For example, a study in Wisconsin (USA) and Europe shows that increased nitrogen deposition inhibits soil microbial activity, soil respiration and the rate of litter decomposition (Berg, 1988; Haynes and Gower, 1995).

Soil microbes are the most active part in terrestrial ecosystems. They decay biological residue, drive nutrient transformation, organic carbon metabolism, contamination and degradation and are important in the soil energy cycle and nutrient transportation (Wang et al., 1998). A variety of soil microbial biomass carbon is important to nutrient cycling in terrestrial ecosystems. Microbial biomass carbon is sensitive to minute changes in total organic carbon in forest soils (Wang et al., 1998). During dry seasons with low soil temperatures and slow plant growth, a decrease in the amount of litter and reduced soil secretion combine

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against the growth of microbes. But during the wet season, vigorous plant growth and more secretion to the underground enhance the activity of microbial metabolism (Yi et al., 2005). Furthermore, soil microbial biomass carbon could be used as an index of the changes of microbial soil activity under nitrogen deposition (Paul and Beauchamp, 1996). In addition, changes of soil microbial activity can impact the release of green house gas and carbon stock into the terrestrial ecosystem (Compton et al., 2004). Hence, investigation into the effects of elevated nitrogen deposition on soil microbial biomass carbon will be important in understanding the effects of nitrogen deposition on underground ecological processes and in the evaluation of carbon sequestration in terrestrial ecosystems.

However, present investigations into the effects of nitrogen deposition on forest soil microbial biomass carbon are focused on North America and few studies in tropical and subtropical regions have been reported so far (Mo et al., 2004). The response of forest soil microbes to nitrogen deposition in tropical and subtropical region would be more intensive than that in temperate zones. In the last 20 years, high levels of nitrogen deposition have occurred in eastern regions of China, largely because of their frantic economic development. For example, N deposition of Guangzhou City was 46–73 kg/(hm²·a) in the latter part of the 1990s (Ren et al., 2000). Nitrogen deposition at the Dinghushan Nature Reserve, located in the under the wind gap of the Zhujiang Delta, Guangdong Province, was 36 kg/(hm²·a) in 1989, and 38 kg/(hm²·a) 10 years later. This amount was equivalent, at the same time, to that in some high nitrogen deposition areas in Europe and North America (Huang et al., 1994; Zhou and Yan, 2001) and over four times that in the Heshan forest (8 kg/(hm²·a)) and the tropical forests of Xishuangbanna (9 kg/(hm²·a)) (Yao and Yu, 1995; Sha et al., 2002). Our country has become one of the three major N deposition regions in the world (Fenn et al., 1998). However, studies on the effects of elevated nitrogen deposition on soil microbial biomass carbon in forest ecosystems in China have not been released.

In our study, we chose a *Pinus massoniana* forest, a mixed forest and an evergreen monsoon broad-leaved forest to examine the response of soil microbial biomass carbon to nitrogen deposition and to investigate the effects of nitrogen deposition on underground ecological processes and evaluate the carbon sequestration in terrestrial ecosystems.

2 Materials and methods

2.1 Study site

Our study was conducted in the Dinghushan Nature Reserve (DHSBR). The reserve lies in the middle of Guangdong Province in southern China (23°08'N, 112°35'E)

and occupies an area of approximately 1200 hm². The mean annual rainfall is 1927 mm. There is a distinct seasonal pattern: 75 per cent of rainfall falls from March to August and only 6 per cent from December to February (Huang and Fan, 1982). The average annual relative humidity is 80%. The mean annual temperature is 20.9°C. The coldest and hottest monthly temperature of 12.6 and 28.0°C occurred in January and July, respectively. The monsoon evergreen broad-leaved (MEBF) forest has been protected by monks in temples for over 400 years (Mo et al., 2003, 2006). The pine broad-leaved mixed forest (MF) originated from pine plantations and were naturally invaded and colonized by broadleaved species. MF is a transitional forest from pine forest to an evergreen monsoon broad-leaved forest. The pine forest (PF) was planted in about 1930. It has been disturbed by human activities ever since it was planted. Thus, these plantations varied in the level of human impacts as well as in their stages of succession, site conditions and species (Mo et al., 2003).

2.2 Plot design

Experiments in nitrogen addition were initiated within each of the three forest types in 2003. Four treatments of nitrogen addition, each replicated three times, were established in mature forests: control (without added N), low N (50 kg N/(hm²·a)), medium N (100 kg N/(hm²·a)) and high N (150 kg N/(hm²·a)), but only three treatments were established in rehabilitated and disturbed forests, i.e., control, low N and medium N. Thirty 20 m by 20 m plots were established, 12 in mature, nine in rehabilitated and nine in disturbed forests, surrounded by a 10 m wide buffer strip. All plots and treatments were laid out randomly. NH₄NO₃ solution was sprayed monthly by hand onto the floor of these plots starting in July, 2003. Fertilizer was weighed, mixed with 20 L of water and applied below the canopy, to the plots using a backpack sprayer. Two passes were made across each plot to ensure an even distribution of the fertilizer. The control plots received 20 L water without nitrogen added (Mo et al., 2004, 2006).

2.3 Sampling and experimental methods

Soil samples were collected in the middle of November, 2004 and in June, 2006, by driving a 2.5 cm diameter auger into the ground from the soil surface to a depth of 10 cm, randomly, at each plot of the pine forest (PF), the pine and broad-leaved mixed forest (MF) and the monsoon evergreen broad-leaved forest (MEBF). The eight soil cores were combined to form a composite soil sample for each plot. These composite samples were gently homogenized and stored at 4°C until processing. Large roots, wood and litter were removed from the composite samples, which were then passed through a 2 mm mesh sieve. Microbial biomass C was based on the difference between dissolved

organic C extracted with 0.5 mol/L K_2SO_4 for 1 h on a shaker from chloroform-fumigated and unfumigated soil samples using a K_c factor of 0.33 (Vance et al., 1987; Jenkinson, 1987). Extractable dissolved organic carbon (DOC) in the K_2SO_4 extracts was analyzed using a total carbon analyzer, Shimadzu model TOC-500. Soil pH and Al^{3+} concentrations were measured by an acidity electrode and spectrum analyzer ICP (Optima-2000) instrument, in September, 2005 (Phillips and Yanai, 2004). Results are presented per unit oven-dry soil at 105°C.

2.4 Statistics

Analyses of variance were carried out to test the difference in microbial biomass carbon and extractable DOC among forest types, seasons and nitrogen treatments, as well as the difference in soil pH and Al^{3+} concentrations among forest types and N treatments. Then, LSD tests were used to compare the difference in microbial biomass carbon, extractable DOC, soil pH and Al^{3+} concentrations among the effects of N treatments. All analyses were conducted using SPSS10.0 for Windows. Statistical significant differences were mostly set at p values < 0.05.

3 Results

3.1 Soil humidity and temperature

In November 2004, soil humidity of PF, MF and MEBF were 10.48%, 9.64% and 18.1% and soil temperatures were 22.65°C, 22.14°C and 19.56°C respectively. In June 2006, the soil humidity of PF, MF and MEBF were 22.16%, 23.78% and 26.54% and the soil temperatures were 28.62°C, 27.66°C and 25.67°C respectively. These results show that soil humidity and soil temperature in June, 2006 were higher than those in November, 2004 and soil humidity in the MEBF was higher than in PF and MF. Analyses of variance indicated that forest types and seasons clearly affected soil humidity and temperature ($p < 0.01$) and their interaction was significant as well ($p < 0.01$). The difference in soil humidity and temperature between the MEBF and MF or PF was significant ($p < 0.01$).

3.2 Soil pH and Al^{3+} concentration

Soil pH in the MEBF was lower than that in MF and PF ($p < 0.05$), but the difference between the MF and PF was not significant (Fig. 1). The Al^{3+} concentration in MEBF was clearly higher than that in the MF and PF ($p < 0.05$), but the difference between the MF and PF was not significant (Fig. 2).

Correlation analysis shows that soil pH was negatively correlated with nitrogen treatments in MEBF ($p < 0.05$, Fig. 1), but correlation between soil pH and nitrogen treatment was not significant in MF and PF. The differences in

soil Al^{3+} concentration among nitrogen treatments were significant in MEBF ($p < 0.05$, Fig. 2), but the differences were not significant in MF or PF. Soil Al^{3+} concentration in MEBF increased with the improvement in nitrogen treatment levels (Fig. 2) and soil Al^{3+} concentration in the high level nitrogen treatment was higher than that of the control treatment ($p < 0.05$).

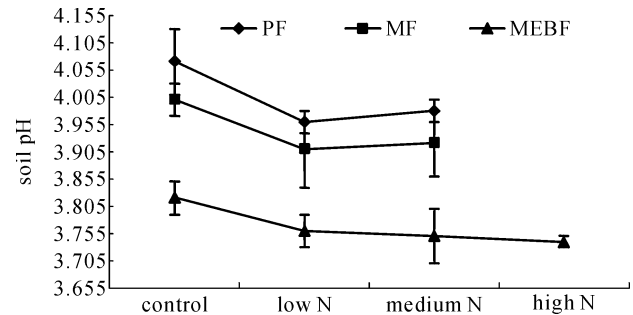


Fig. 1 Soil pH in forests with different levels of N treatment

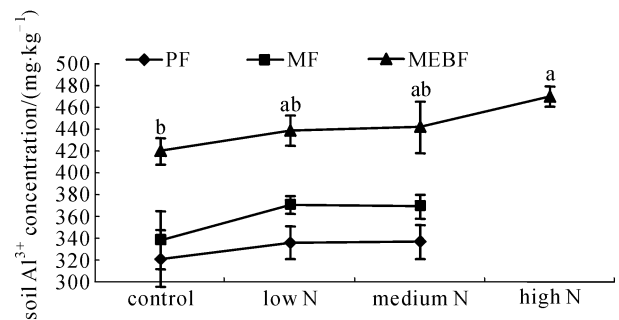


Fig. 2 Soil Al^{3+} concentration in forests with different levels of N treatment

Note: different small letters means significant difference at 5% level. The same comments apply to Figs. 3 and 4.

3.3 Soil microbial biomass carbon and extractable DOC in control treatment

Soil microbial biomass carbon and extractable DOC in June, 2006 were higher than those in November, 2004 in the MEBF, MF and PF ($p < 0.05$). During the same sampling time, soil microbial biomass carbon and extractable DOC in the MEBF were also clearly higher than those in the MF and PF ($p < 0.05$), but the difference between the MF and PF was not significant (Figs. 3 and 4). Moreover, interactions of soil microbial biomass carbon and extractable DOC among sampling dates and forest types were not clear.

3.4 Effects of elevated nitrogen deposition on soil microbial biomass carbon and extractable DOC

Analyses of variance indicated that the differences in soil microbial biomass carbon and extractable DOC between two sampling dates was neither significant in the PF nor in

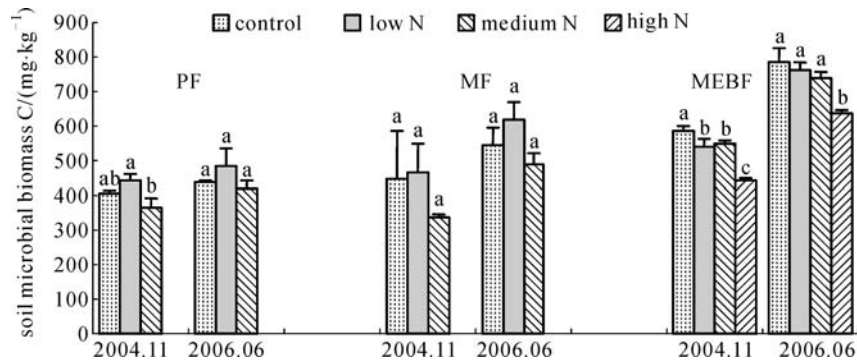


Fig. 3 Soil microbial biomass C in forests with different levels of N treatment

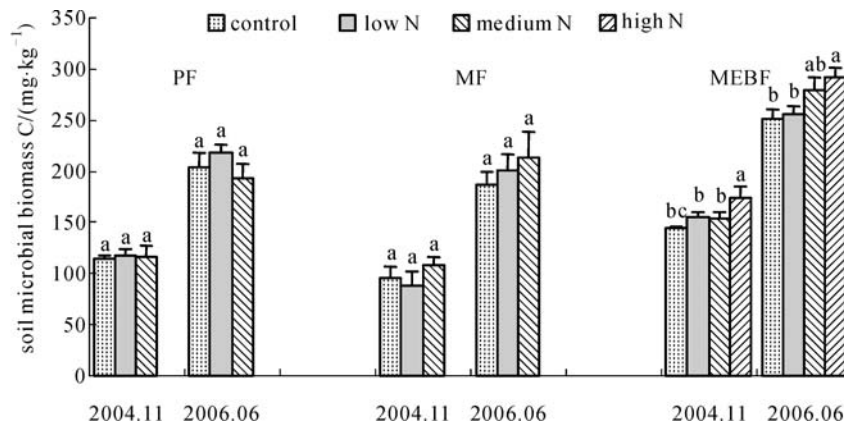


Fig. 4 Soil extractable DOC in forests with different levels of N treatment

the MF, but in the MEBF the differences were significant ($p < 0.05$). LSD tests show that soil microbial biomass carbon decreased with increased nitrogen treatment levels (Fig. 3). Soil microbial biomass carbon in high level nitrogen treatment was lower than that in the control treatment on the two sampling dates ($p < 0.05$). In contrast, extractable DOC increased with improved nitrogen treatment levels (Fig. 4). Extractable DOC in high level nitrogen treatment was higher than that in the control treatment on both sampling dates ($p < 0.05$).

4 Discussion and conclusions

4.1 Effects of forest type and season on soil microbial biomass C and extractable DOC

In our study, soil microbial biomass C and extractable DOC in the MEBF were higher than those in the MF and PF, but there were no significant differences between the MF and PF (Figs. 3 and 4), which is due to the difference of available nutrient for soil microbes, dominated by the variety of litter and the rate of decomposition. As in the previous analysis, for the three forests, the variety of litter in MEBF was greater than that in the MF and PF. The rate

of litter decomposition in the MEBF was faster than that in the MF and PF (Mo et al., 2004, 2006). Soil microbial biomass, therefore, was higher in the MEBF, due to the greater amounts of available nutrients for soil microbes in this forest. Our results agree with those of previous investigations in Dinghushan (Fu et al., 1995; Yi et al., 2005).

The seasonal variation in the rate of litter decomposition resulted in higher soil microbial biomass C and extractable DOC in June, 2006 than that in November, 2004. From the long-term climate data, it can be observed that the wet season is from April to September and the dry season from October to March. Temperature begins to increase in March to May and the highest temperatures occur in July and August (Yi et al., 2005). In our study, humidity and temperature in the wet season were all higher than in the dry season. The period of the highest temperature and humidity coincided with the phase of the fastest rate of litter decomposition and microbial activity. The assimilation rate of nutrients for soil microbes was fast in a high temperature and humidity environment. Soil microbial biomass C and soil extractable DOC, therefore, are higher. That was consistent with the results from Yi et al. (2005) on forest soil microbial biomass by chloroform incubation. Some other studies also gave similar results. Compton et al.

(2004) indicated that soil microbial biomass C and extractable DOC in June 2001 were higher than those in November 2000 in the Harvard forest. Acea and Carballas (1990) and Diaz-Ravina et al. (1995) also concluded that the soil microbial biomass in wet season was higher than that in dry season.

4.2 Effects of N deposition on soil microbial biomass C

High nitrogen treatment decreased soil microbial biomass C in the MEBF but increased the soil extractable DOC. The potential factors were as follows.

1) Soil acidification of nitrogen treatment could be due to the augmentation of available soil nitrogen (Fang et al., 2006). Incremental amounts of $\text{NH}_4^+\text{-N}$, absorbed by plants, and translated into $\text{NO}_3^-\text{-N}$ by nitrobacteria or eluviated out from the soil, increases the H^+ concentration in forest ecosystems (Matson et al., 1999). Nitrogen also enhanced $\text{NO}_3^-\text{-N}$ concentration in soil. While the excess $\text{NO}_3^-\text{-N}$ was leached out from the soil, soil acidification occurred (Katzensteiner et al., 1992). The soil microbial community was inhibited by soil acidification (Baath et al., 1980). Joergensen et al. (1995) indicated that soil microbial biomass decreased when soil pH was less than 5 in their soil acidification grade experiment in Germany. Smolander et al. (1994) had shown that, although the soil organic matter increased with a declining soil pH, the soil organic matter was not available for soil microbes, which resulted in a change of soil microbial community structure and the decline of soil microbial biomass. Soil pH was negatively correlated with nitrogen treatment in the MEBF (Fig. 1), but not correlated with nitrogen treatment in the PF and MF. However, there was a tendency for soil pH to decline with increased levels of nitrogen treatment. With this increase in the level of N treatment, the soil pH in the PF and MF may show a similar trend as in the MEBF. Soil acidification due to nitrogen deposition would be an important factor that dominates soil microbial biomass.

2) Ca^{2+} , Na^+ and Mg^{2+} were eluviated from the soil with additional nitrogen, which led to the aggravated soil acidification (Foster et al., 2004). The effect of aluminium toxicity can induce poor nutrient conditions around plant roots. The decline of plant root growth and activity and an inhibition of rhizosphere microorganisms prevent the formation of soil microbial biomass. Li (1997) found soil pH and roots biomass clearly decreased when Al^{3+} was added, which jeopardized the growth of rhizosphere microorganisms. Nitrobacteria, denitrifying bacteria and soil respiration also decreased with the addition of Al^{3+} (Luo et al., 2004). In our study, Al^{3+} concentration increased with the addition of N in the MEBF (Fig. 2). The decrease of soil microbial biomass indicated the presence of aluminium toxicity. The threshold of aluminium toxicity to soil microbes needs further study.

3) The status of available nitrogen was amended by

additional input of nitrogen into the forest ecosystem. Plants decrease the growth of roots and the release of exudates, when the underground resource allocation declines (Wallenstein et al., 2006). Therefore, the microorganisms of the rhizosphere, which depend on nutrients from root exudates, would be inhibited and soil microbial biomass should decline (Wallenstein et al., 2006). The addition of nitrogen could change the net of soil mycelium, mycorrhiza formation and community structure of mycorrhizal fungi (Wallenda and Kottke, 1998; Lilleskov et al., 2002; Frey et al., 2004). Moreover, changes in the chemical components of litter and competitive relations between plants and soil microbes, affected soil microbial biomass with their nitrogen deposition (Wallenstein et al., 2006). Based on our study, we predict the decline of soil microbial biomass of this ecosystem after several years of nitrogen addition to the MEBF. The response of soil microbial biomass to nitrogen deposition is coupled with the processes of aboveground forest biomass and litter decomposition. With the slowing of litter decomposition and supply of soil organic matter, elevated levels of nitrogen deposition would inhibit soil microbial biomass in the greater time scale (Compton et al., 2004).

Our results agree with previous research. Xue et al. (2007) found elevated nitrogen levels of deposition decreased soil microbial growth in a nursery adjacent to the forest, as we found in our study. Many studies also indicated that nitrogen addition significantly decreased soil microbial biomass in Europe and North America (Arnebrant et al., 1990; Wallenstein, 2003; Compton et al., 2004; Frey et al., 2004).

4.3 Effects of N deposition on forest soil organic carbon sequestration

Soil dissolved organic carbon is considered to be the most dynamic compound of soil carbon and is the main source of energy and material of a microbial soil community (Matlou and Haynes, 2006). Dissolved soil organic carbon also includes recalcitrant aromatic carbon molecules, which are difficult to decay by soil microbes (Guggenberger and Zech, 1994). Hence, dissolved soil organic carbon is not only the substrate of soil microbial activity but also the main products of soil microbial metabolism. Therefore, dissolved soil organic carbon is intensively related to soil microbial biomass (Matlou and Haynes, 2006). Piao et al. (2000) found soil microbial biomass was negatively correlated with extractable DOC by K_2SO_4 solution.

Mo et al. (2004, 2006) had shown that nitrogen addition significantly inhibited litter decomposition in the MEBF, which confirms that extractable DOC increases with the addition of nitrogen to the MEBF. These results show that nitrogen addition may enhance forest soil carbon sequestration. Bowden et al. (2004) found that nitrogen addition decreased soil microbial biomass and soil CO_2 efflux.

Deforest et al. (2004) had shown that the dissolved organic carbon increased with phenolic matter accumulation due to N addition. Magill et al. (1998) also found that N addition resulted in soil organic carbon accumulation by inhibiting litter decomposition.

However, we also found that soil microbial biomass carbon and extractable DOC were affected by nitrogen addition in the MF and PF. That could be due to the timing of the addition of nitrogen and to soil nitrogen conditions. Soil microbial biomass was impacted by the addition of N at least six years after an elevated nitrogen treatment in temperate ecosystems. Nitrogen addition would not affect soil microbial biomass when the ecosystem was at an early stage of nitrogen saturation (Wallenstein et al., 2006). In our study, the higher N status in the MEBF led to the condition that soil microbial biomass was affected after three years of nitrogen addition (Mo et al., 2004, 2006).

5 Conclusions

Soil microbial biomass C and extractable DOC were higher in the wet season than during the dry season and higher in the MEBF than in the PF or MF. In the PF or MF forests, no significant effects of nitrogen addition were found on soil microbial biomass C and extractable DOC. In the MEBF forest, however, soil microbial biomass C generally decreased with increasing levels of nitrogen. The response of soil extractable DOC to nitrogen addition in the MEBF shows opposite trends to soil microbial biomass C. These results suggest that nitrogen deposition may increase the accumulation of soil organic carbon in MEBF in the study region.

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